PATENT COOPERATION TREATY

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NOTIFICATION OF TRANSMITTAL OF COPIES OF TRANSLATION OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 72.2)

From the INTERNATIONAL BUREAU

To:

ROCHE DIAGNOSTICS GMBH
Patentabteilung
D-68298 Mannheim
ALLEMAGNE

Date of mailing (day/month/year) 30 January 2002 (30.01.02)	
Applicant's or agent's file reference 5181/OA/WO-lm	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/00602	**International filing date** (day/month/year) 27 January 2000 (27.01.00)
Applicant ROCHE DIAGNOSTICS GMBH et al	

1. Transmittal of the translation to the applicant.

The International Bureau transmits herewith a copy of the English translation made by the International Bureau of the international preliminary examination report established by the International Preliminary Examining Authority.

2. Transmittal of the copy of the translation to the elected Offices.

The International Bureau notifies the applicant that copies of that translation have been transmitted to the following elected Offices requiring such translation:

AU,CA,CN,JP,KR,NZ,US

The following elected Offices, having waived the requirement for such a transmittal at this time, will receive copies of that translation from the International Bureau only upon their request:

EP,HU,IL,MX,NO,PL,RU,ZA

3. Reminder regarding translation into (one of) the official language(s) of the elected Office(s).

The applicant is reminded that, where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report.

It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned (Rule 74.1). See Volume II of the PCT Applicant's Guide for further details.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 5181/OA/WO-Im	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP00/00602	International filing date (*day/month/year*) 27 January 2000 (27.01.00)	Priority date (*day/month/year*) 29 January 1999 (29.01.99)
International Patent Classification (IPC) or national classification and IPC G01N 33/68		
Applicant ROCHE DIAGNOSTICS GMBH		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 25 May 2000 (25.05.00)	Date of completion of this report 10 May 2001 (10.05.2001)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP00/00602

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-24, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the claims:

pages _____, as originally filed

pages _____, as amended (together with any statement under Article 19

pages _____, filed with the demand

pages 1-19, filed with the letter of 03 January 2001 (03.01.2001)

☐ the drawings:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☐ the sequence listing part of the description:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5 - 19	YES
	Claims	1 - 4	NO
Inventive step (IS)	Claims		YES
	Claims	1 - 19	NO
Industrial applicability (IA)	Claims	1 - 19	YES
	Claims		NO

2. Citations and explanations

Reference is made to the following documents:

D1: WO-A-93/24531
D2: WO-A-89/12069
D3: Clin. Endocrinol. (1997) **47** 287-296

1. Novelty (PCT Article 33(2)):

1.1 The subject matter of **Claim 1** is anticipated by D1 (D1: Claim 9). D1 describes in detail the production of the monoclonal antibody 1C7 (Example 1; pages 7-8). The method described, including the cloning, can be reproduced by a person skilled in the art. Accordingly, a person skilled in the art is also able to produce a second antibody directed against BNP(1-76), which then also enables a sandwich assay to be carried out (D1: page 9, paragraph 1; Claims 7-9). Although the antibodies of the present application produced by peptides (application: page 20, Table 1) do not detect native pro-BNP, this does not mean that the antibodies produced as per D1 (Example 1) likewise do not detect the native pro-BNP since other peptides are used in D1 (BNP(1-21), BNP(22-46), BNP(47-64)).

Therefore the novelty of **Claims 2 to 4** is also anticipated by D1.

1.2 **Claim 5** is novel since none of the available documents discloses a method which uses two antibodies that detect different antigenic determinants of N-terminal proBNP and have a detection limit of less than 1fmol/ml patient blood (= 1 pmol/l).

1.3 **Claim 6** is novel since none of the available documents discloses a BNP-based detection method which permits differentiation between healthy patients and those suffering cardiac insufficiency of NYHA Classes I - IV. Accordingly dependent **Claim 7** is likewise novel; the same applies to independent **use Claim 8.**

1.4 **Claim 9** is novel since none of the available documents discloses recombinant N-terminal proBNP. Accordingly the use of recombinant N-terminal proBNP (**Claims 10 and 11**), specific antibodies (**Claims 12 to 16**), their production (**Claims 18 and 19**) and the associated cell lines (**Claim 17**) are also novel.

2. Inventive step (PCT Article 33(3)):

2.1 The single additional technical feature in **Claim 5** concerns the result to be attained with the invention, namely a low N-terminal proBNP detection limit (< 1 fmol/ml). Since the method of detecting N-terminal proBNP in a sample (Claim 1) is not novel, the claimed detection limit appears to be attainable by normal experimentation with specific

antibodies (PCT Article 33(3)).

2.2 **Claim 6** does not appear to be inventive:
D3, which is considered the closest prior art, specifies that the N-terminal proBNP (NT-proBNP) can be used as a cardiac insufficiency marker. The NT-proBNP level in NYHA Class 1 patients is higher than in healthy patients and increases as the cardiac insufficiency increases, the D3 assay permitting differentiation between healthy patients and NYHA Class I - IV patients (D3: page 287, column 2, paragraphs 2, 3; page 291, Figure 3). The method for determining NT-proBNP which is used in D3 is a radio immunoassay that uses an antiserum produced to act against human proBNP (1-13) (D3: page 288, column 2, paragraph 1). Consequently D3 differs from the subject matter of Claim 6 by the detection method. Whilst two antibodies detecting different antigenic determinants on N-terminal proBNP are used according to Claim 6, the D3 method uses only one antibody in a competitive test format (D3: page 288, column 2, paragraph 1).

The object achieved in Claim 6 is that of preparing a sensitive assay which only requires short incubation periods (see application, page 4, paragraph 3).

A person skilled in the art is familiar with sandwich assays with two antigen-specific antibodies (e.g. D1: Claim 9; page 8, last line - page 9, paragraph 1), and with their advantages such as, for example, the shorter incubation periods (D1: page 9, paragraph 1). Accordingly the teaching of D3, that is, the use of NT-proBNP as marker for

distinguishing between healthy plasma and NYHA Class I - IV plasma in conjunction with a sandwich ELISA appears to suggest the subject matter of Claim 6. Therefore **Claims 7 and 8** likewise appear to be non-inventive.

2.3 **Claim 9** does not appear to comply with PCT Article 33(3). The significance of N-terminal proBNP as diagnostic indicator or predictor for cardiac insufficiency has been known for a long time (e.g. D1: page 3, lines 2-6) and the use of antibodies against the N-terminal proBNP peptide in immunoassays in particular is described in the prior art (e.g. D1: page 3, line 7 - page 5, line 11). D1, which is considered the closest prior art, describes the production (D1: page 6, paragraph 3) of proBNP (1-76) by chemical synthesis, which is a long-known standard method of producing peptides. Moreover, D1 describes the use of N-terminal proBNP(1-76) for producing antibodies (D1: Claim 15). However, D1 does not describe the production and use of recombinant BNP(1-76).

The object achieved in Claim 9 is consequently that of devising an alternative method for producing a proBNP(1-76) peptide. D2 discloses the cDNA sequence of human BNP. Figure 5 (D2: page 16, lines 24-27) discloses the DNA sequence of human BNP (coding region of the plasmid phBNP-1) and the associated peptide sequence. Example 5 (page 41) of D2 describes the cloning of human BNP. Moreover, D2 describes the production of BNP in recombinant expression systems (D2: page 20, line 18 - page 26, line 25).

The significance and use of N-terminal proBNP is described in D1.

Furthermore, the production of a recombinant protein on the basis of a known cDNA sequence is a standard technique for a person skilled in the art.

In conclusion, the sequence disclosed in D2 in combination with the significance and use described in D1 appears to render the subject matter of **Claim 9** obvious.

Since the production of antibodies with peptides and proteins is likewise a familiar technique for a person skilled in the art and is also described, for example, in D1 (pages 7-8), **Claims 12, 14-16, 18** (D1: page 9, paragraph 2) and **19** (D1: page 7, paragraph 2 - page 8, paragraph 3) likewise appear not to comply with PCT Article 33(3).

Claim 13 concerns antibodies against the 10-66 range of amino acids of N-terminal proBNP. The choice of this range is intended to ensure that the analyte can also be detected when the N or C-terminal amino acids have already been digested by proteases. D1 can be cited against this since it describes the monoclonal antibody 1C7 which specifically binds to the BNP(47-64) sequence. Moreover, the D1 method can also produce an antibody which is directed against the BNP(22-46) peptide (D1: page 7, Example 1, paragraph 1). Therefore Claim 13 appears to be lacking an inherent inventive concept.

The same applies to **Claims 15 to 17**, which concern specific deposited antibodies and the producing cell

lines.

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Contrary to the requirements of PCT Rule 5.1(a)(ii), the description did not cite D2 and it did not briefly outline the relevant prior art contained therein.